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METABOLISM OF PLUTONIUM IN THE RAT

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ABSTRACT

This report gives a comprehensive summary of the project literature dealing with the absorption, excretion, and body deposition of plutonium when administered in various forms and by various routes to the rat.

Additional experimental data are presented regarding the metabolism of plutonium when administered to the rat by intravenous injection. The results indicated the following:

1) The first day following intravenous injection of $PuO_2(NO_3)_2$, the urinary excretion of plutonium was 7.5 per cent of the dose as compared to 0.33, 0.57, and 0.71 per cent when administered as $PuCl_3$, $Pu(NO_3)_4$, and Pu^{+4} citrate complex, respectively. Fecal excretion during the first day was correspondingly lower following injection of $PuO_2(NO_3)_2$. On the thirtieth day following intravenous injection there were no significant differences in either urinary or fecal excretion of plutonium administered as $PuCl_3$, $Pu(NO_3)_4$, Pu^{+4} citrate complex, and $PuO_2(NO_3)_2$. At this time the average urinary excretion was 0.014 per cent of the injected dose and the average fecal excretion was 0.22 per cent. The average ratio of fecal to urinary excretion was 16/1. Urinary and fecal excretion curves are given for all forms of plutonium injected.

2) The skeleton was the principal site of deposition regardless of the form in which the plutonium was injected. Four days following injection of plutonium as $PuCl_3$, $Pu(NO_3)_4$, Pu^{+4} citrate complex, and $PuO_2(NO_3)_2$ skeletal deposition was 44.9, 29.3, 56.9, and 56.5 per cent of the injected dose, respectively. Deposition in the liver under the above conditions was 22.9, 39.7, 9.6, and 9.1 per cent of the injected dose, respectively. Deposition of plutonium in kidney, spleen, and in "balance" was not greatly affected by the form in which the plutonium was administered.

3) The size of the injected dose of Pu^{+4} citrate complex did not affect the per cent of the dose excreted in the feces and urine. Likewise, the size of dose did not alter the per cent of injected material present in the various tissues six days following injection.

4) When Pu^{+4} was administered orally in 5 per cent sodium citrate solution, the presence of the citrate seemed to increase absorption of the plutonium from the gastro-intestinal tract. Absorption was still quite low—only 0.3 per cent of the administered dose.

Seventy-eight per cent of the plutonium absorbed from the gastro-intestinal tract was deposited in the skeleton and 7.3 per cent in the liver. Body deposition of plutonium absorbed via this route seems to resemble that of plutonium injected intravenously as $PuO_2(NO_3)_2$ or Pu^{+4} citrate complex.

* * * * *

INTRODUCTION

Large-scale processing of plutonium was necessary in the development and production of the atomic bomb. The future will undoubtedly see the development of other uses for this material. The toxic nature of plutonium has made the study of its metabolism in the animal organism a matter of great importance. Extensive animal experimentation is imperative if a full understanding of the toxic properties of plutonium is to be gained and if suitable means are to be devised for the protection of the health of persons working with this material.

The rat has been used extensively at this and other laboratories for the study of the metabolism of plutonium The results of many of these studies are scattered through the project literature in the form of preliminary reports. The purpose of this report is to summarize the work on metabolic studies of plutonium and to report additional investigations carried on at this laboratory. These investigations constitute a rather intensive study of the metabolism of this material when administered intravenously to the rat.

Effect of Valence State and Method of Administration on the Absorption of Plutonium by the Rat

The absorption of plutonium into the animal body from the site of administration is greatly affected by the method of administration and the valence state of the plutonium. Hamilton's results (CN-2383) indicate that a solution of PuO_2^{+2} is most readily absorbed, Pu^{+3} next, and Pu^{+4} least. This order seems more or less independent of the method of administration. Table 1 summarizes his results on the effect of valence on the absorption of plutonium when administered intramuscularly, subcutaneously, and intrapulmonarily to rats. The rate of absorption is very probably directly dependent on the solubility of the plutonium compound formed by hydrolysis at the pH of the tissue fluids or absorbing surfaces. That absorption from the site of administration is related to hydrolysis and solubility of plutonium at the absorbing surfaces is indicated by results obtained by the Chicago group (AM-1653). They found that the absorption of Pu^{+4} and PuO_2^{+2} from the lung was greatly enhanced by the presence of citrate ion. Not only was absorption of both valence states increased but both were absorbed to the same degree in the presence of a complexing agent (CN-2740).

Table 1. Effect of valence state on absorption of plutonium from the site of administration.

Method of administration	Valence state	Days after administration and % remaining unabsorbed at site		
		4 days	16 days	64 days
Intramuscular	+3	77	68	39
	+4	96	88	68
	+6	70	29	35
Subcutaneous	+4	--	71	--
	+6	--	42	--
Intrapulmonary*	+3	45	--	--
	+4	94	--	--
	+6	32	--	--

* The administered dose minus the amount remaining in the lung is not a measure of the amount absorbed. Some material was removed from the lung by ciliary action.

The California group made an intensive study of the rate of absorption and excretion of plutonium oxide smokes from the lungs of rats. The PuO_2 smokes were produced in two ways. In one case solutions of $Pu(NO_3)_4$ or $PuCl_4$ were evaporated on carbon electrodes after which the electrodes were arced. The other procedure was to embed small pellets of plutonium metal in carbon electrodes which were then arced. There seemed to be little difference in the behavior of the oxide smokes in the lung when produced by these procedures. In general, their results (CN-2132, CN-2238, CN-2312, CN-2546, CN-2905, CN-3076, AM-1808) indicate a number of important points regarding the behavior of finely divided PuO_2 in the lung of the rat. Animals sacrificed immediately after exposure retained about 80 per cent of the inhaled dose. About 50 per cent of the inhaled material was retained in the upper respiratory tract. In 96 hours the majority of the PuO_2 deposited in the upper respiratory tract was removed by ciliary action, swallowed, and eliminated in the feces. About 20 to 40 per cent of the inhaled dose was deposited in the alveolar areas of the lung where, according to lung radioautographs, it was rather uniformly distributed. At 64 days 10 to 25 per cent of the inhaled activity remained in the lung and at 256 days about 5 per cent remained. At 64 days the rate of bronchial excretion of the plutonium remaining in the lungs was about one per cent per day. They concluded that the half-life of PuO_2 in the lung was about six months.

The absorption of plutonium deposited in the lung as PuO_2 was very slight. Only about one per cent of the inhaled dose was found in the skeleton and other tissues 64 days after exposure. At 129 days they reported the highest per gram activity of tissue to lung was 1/800. The absorption of plutonium from the lung when the plutonium is administered as PuO_2 smoke is much less than the absorption when it is administered as a solution of a soluble salt. These results strongly indicate that exposure to PuO_2 dusts may result primarily in hazard to the lung tissue rather than to the skeletal structures. A urine analysis for plutonium may not be of appreciable value in indicating deposition of PuO_2 in the lung, since its absorption from this region is so extremely low. It may be necessary to develop other methods of diagnosing exposure in cases where persons are in contact with PuO_2 dust.

Orally administered plutonium is absorbed very slightly by the rat. Hamilton et al (CN-2383) reported that less than 0.05 per cent of the administered dose was absorbed from the gastro-intestinal tract regardless of valence state. In a later study (MUC RRS 553) they found that a series of 4 rats showed an average skeletal uptake of 0.006 per cent of an orally administered dose of 45γ of $PuO_2(NO_3)_2$. If the skeletal deposition of plutonium is taken as 60 per cent of the absorbed dose, then the absorption of orally administered plutonium would be about 0.01 per cent.

The Chicago group reported (MUC KSC 520) that the mouse absorbed 0.06 per cent of a 6.15-mg dose of plutonium administered orally as $Pu(NO_3)_4$. They found the body distribution of plutonium absorbed from the gastro-intestinal tract was essentially the same as that absorbed from other sites of administration.

We are presenting in this report additional information on the body distribution of plutonium absorbed from the gastro-intestinal tract of the rat.

Effect of Valence State and Method of Administration on Distribution of Plutonium in the Body

Hamilton and co-workers (CN-2383) studied the metabolism of Pu^{+3}, Pu^{+4}, and PuO_2^{+2} when administered subcutaneously, intramuscularly, intrapulmonarily, and intravenously to rats. They found that regardless of the valence state of the plutonium and the route of administration, the body deposition was essentially the same when correction was made for the amount of material remaining unabsorbed at the site of administration. A possible exception to this general statement is the retention of plutonium by the liver following intravenous injection. The California group found 38, 73, and 29 per cent retention of plutonium in the liver four days after intravenous injection of Pu^{+3}, Pu^{+4}, and PuO_2^{+2}, respectively. The respective amounts of absorbed plutonium retained in the liver four days after intramuscular administration were 5.5, 3.5, and 14 per cent. The high liver retention following intravenous injection was explained by Hamilton (CN-2383) as possibly resulting from precipitation of the product in the blood and filtering out of the particles by the reticulo-endothelium. The Chicago Laboratory apparently does not agree with this postulation (CN-2653).

There also seems to be considerable disagreement regarding the amount of plutonium retained in the liver of the rat following intravenous injection of PuO_2^{+2}. Hamilton found only 3 per cent of the plutonium was retained in the liver 16 days after injection although he had reported earlier that 29 per cent was retained at the end of the fourth day. The Chicago Laboratory found from 20 to 40 per cent of the plutonium deposited in the livers of both rats and mice 4 to 14 days after intravenous injection of PuO_2^{+2}, even in the presence of complexing agents, i.e. citrate ion (CN-2905).

Results obtained at California, Chicago, and in this laboratory show conclusively that the skeletal system is the principal site of plutonium deposition in the body. More than 50 per cent of the absorbed dose seems to deposit in the skeleton regardless of valence state of the plutonium or the method of administration. A possible exception, of course, may be the case of unusually high liver deposition following intravenous injection of Pu^{+4}. Radioautographic studies by both the California (CN-2905) and Chicago groups (CN-2312) show that the site of plutonium deposition within the skeleton is in the endostium and periostium. Deposition is especially high in the trabecular region. Excellent studies by Copp and Hamilton (MUC RSS-540) have shown conclusively that plutonium is fixed in the osteoid matrix and not in the bone salts. Their studies showed that plutonium concentrated in the matrix of the callus surrounding a fracture. If it was not administered to the animal until bone salts had invaded the callus, concentration of plutonium in the callus was slight. They also showed that it was possible to trap a major part of the plutonium deposited in the endostium beneath a layer of bone salts by means of a decalcification-calcification cycle. The best results were obtained when the animal was decalcified by being kept on a phosphorus-free diet.

The kidney and spleen retain relatively large amounts of plutonium although their specific activity is only about one-fifth to one-tenth that of bone. The kidney, spleen, and liver retain about 0.5 to 1.0 per cent per gram of the absorbed plutonium four days after administration. The retention in these organs seems unaffected by the valence state of the plutonium and the method of administration. An exception to this statement is the case of high retention by the liver and spleen following intravenous injection of Pu^{+3} and Pu^{+4}.

The plutonium content of nerve, muscle, and reproductive tissues is quite low. The extremely low retention by these tissues also seems unaffected by valence state of plutonium and by method of administration.

The effect of valence state and method of administration on the concentration and distribution of plutonium in the blood of rats has not been studied very thoroughly. Russell et al (CN-3167) (CN-2653) have made a detailed study of the concentration and distribution of plutonium in the blood of the dog following intravenous administration of a lethal dose of $PuO_2(NO_3)_2$ — 0.36 mg per kg. The plutonium concentration in the blood decreased tenfold in the first half hour and after seventeen days reached an equilibrium concentration of about 0.01γ per ml of blood. Practically all of the plutonium in the blood was contained in the β-globulin fraction of the plasma.

Effect of Valence State and Method of Administration on the Excretion of Plutonium by the Rat

The California group have reported studies of the effect of valence state and method of administration on the rate of excretion of plutonium (CN-2383). They stated that there was no apparent significant difference in the excretion of plutonium in its three valence states. The data that they present to support their conclusion are rather limited, however. From their data it seems that the ratio of fecal to urinary excretion was from 5/1 to 10/1. This was true for plutonium in all three valence states four days after either intravenous or intramuscular administration provided correction was made for the amount of plutonium remaining unabsorbed at the site of the intramuscular injection. The ratio of fecal to urinary excretion of absorbed plutonium administered as Pu^{+4} and PuO_2^{+2} was essentially 10/1 during the first 16 days following subcutaneous injection.

The Chicago laboratory followed the excretion of plutonium by a group of 5 rats given intravenous injections of lethal doses of $PuO_2(NO_3)_2$ (CN-2905). During the first 14 days, 14 per cent of the injected

dose was excreted. The ratio of fecal to urinary excretion was 3/1. In another group of rats given $PuO_2(NO_3)_2$ intravenously, they found the plateau of urinary excretion occurred at about 25 days. At this time the animals were excreting 0.01 to 0.02 per cent of the injected dose per day. Fecal excretion did not level off until about 40 days. The daily fecal excretion of plutonium at 40 days was about 0.08 per cent of the injected dose. The results of these studies have been summarized in tabular form by E. R. Russell (CN-3167).

EXPERIMENTAL METHODS

General Experimental Procedure

The purpose of the experiments conducted at this laboratory was to study in detail the effect of citrate ion, valence state, and various other factors on the body distribution and excretion of plutonium when administered intravenously to the rat.

Young male rats ranging in size from 200 to 300 g were used for the experiment. The animals were arranged into four groups of 12 rats each. Each animal was injected intravenously with approximately 15 γ of plutonium. The rats in the first group received plutonium in the form of a solution of $PuCl_3$, the second group received $Pu(NO_3)_4$, the third group received $PuO_2(NO_3)_2$, and the fourth group received Pu^{+4} complexed with sodium citrate present as 0.3 per cent $Na_3C_6H_5O_7 \cdot 5\frac{1}{2}H_2O$.

The animals in each group were housed three to the cage. The metabolism cages were specially constructed to facilitate the collection of urine and feces separately. The cage arrangement was essentially that used at the University of Rochester School of Medicine. The details of the arrangement are shown in Figure 1.

Urine and feces samples were collected at 24-hour intervals through the sixteenth day after which they were collected at 48-hour intervals. Each sample consisted of the pooled excretion from three rats. Feces samples were dried immediately at 105°C and stored. Urine samples were collected without the use of a preservative and stored in the refrigerator.

In order to determine body deposition of the injected plutonium, one-third of the animals in each group were killed and autopsied at one of the following time intervals, 4 days, 16 days, or 32 or 48 days after injection. Liver, kidneys, spleen, and carcass were saved separately for analysis. All samples were preserved by placing them in suitable screwcapped specimen jars and desiccating in a drying oven at 105°C for 48 hours. The jars were sealed and the samples stored in the refrigerator.

Preparation of Solutions

The solution of $PuCl_3$ used in these experiments was prepared as follows: A piece of plutonium metal was placed in a volumetric flask and dissolved in the smallest possible amount of 6N HCl by adding the acid dropwise from a micropipet. When the metal was completely dissolved, enough 6N HCl was added to make the solution 1N with acid when diluted to volume with water. This solution was assayed for Pu by radioassay.

An injection solution of $PuCl_3$ containing 45 γ Pu per ml was prepared from this stock solution by diluting a suitable aliquot to the desired concentration with water. This solution had a pH of approximately 2 upon final dilution.

The $Pu(NO_3)_4$ stock solution was prepared from an aliquot of the $PuCl_3$ stock. Enough 8N HNO_3 was added to make the solution 1N in acid when diluted to volume. Before diluting to volume, however, the solution was allowed to stand until the blue color of Pu^{+3} had completely changed to the greenish brown of $Pu(NO_3)_4$. After dilution, the plutonium concentration was determined by radioassay. A complete spectral absorption curve was run on the solution to establish the complete absence of Pu^{+3} and PuO_2^{+2}. The solution to be injected was prepared from this stock by diluting a suitable aliquot to the proper concentration with distilled water. The solution was prepared just before use. It had a pH of 2 and contained 45 γ Pu per ml.

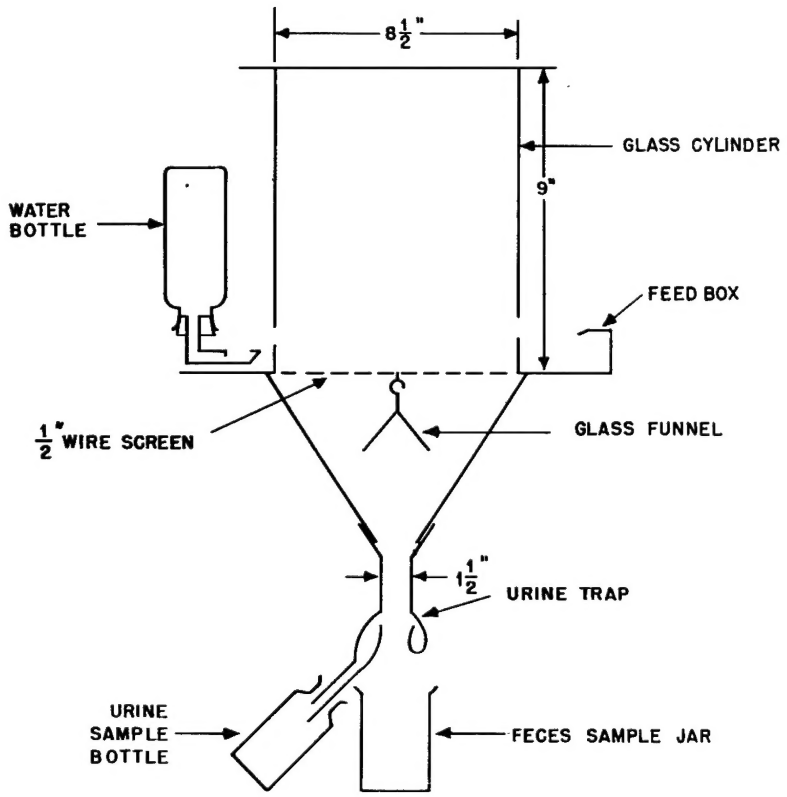

Figure 1. Metabolism cage assembly.

The Pu^{+4} citrate solution used for injection was prepared from the $Pu(NO_3)_4$ stock solution by diluting a suitable aliquot to the desired plutonium concentration using a solution containing 0.3 per cent $Na_3C_6H_5O_7 \cdot 5\frac{1}{2}H_2O$ to make the dilution. The final solution contained 45 γ Pu per ml and had a pH of 4.5.

A stock solution of $PuO_2(NO_3)_2$ was prepared from the +4 nitrate. The desired amount of $Pu(NO_3)_4$ in 1N HNO_3 was transferred to a volumetric flask. Enough $NaBrO_3$ was added to make the solution 0.05M upon final dilution. After standing for 2 hours the solution was diluted to volume with 1N HNO_3.

An absorption curve was run on this solution. There was no indication of the presence of Pu^{+3} and Pu^{+4}. The injection solution was prepared just before use by diluting the stock solution to the desired plutonium concentration with distilled water. A sample of the final solution was assayed for the presence of Pu^{+3} and Pu^{+4} using a basic cupferron extraction in the presence of citrate and dichromate ions, as proposed by Fryxell (LA-395). Over 97 per cent of the plutonium in the final solution was in the plutonyl state. The pH of the solution was approximately 2 at the time of the injection.

Method of Injection

All injections were made by way of the femoral vein. The rat was anesthetized with ether. A half-inch incision was made in the skin over the region of the femoral vein. The vein was exposed and the fascia carefully cleared away. While one operator held pressure on the vein above the point of

injection, another operator carefully entered the vein with a 5/8-inch, 27-gauge hypodermic needle bearing a 1-ml tuberculin syringe filled to the 1/2-ml mark with the solution to be injected. The needle was extended about 1/4 inch into the vein toward the heart. Pressure on the vein was released and approximately 15 γ of Pu slowly injected. The volume of solution injected was approximately 0.35 ml in all cases.

The actual amount of plutonium injected was determined by making "dummy" injections as follows: During the injection of a particular group of rats an amount of solution equivalent to that administered to each rat was discharged from the syringe through the same needle used for the injection into each of five 50-ml volumetric flasks. After dilution to exactly 50 ml with 4N HCl, the solutions were assayed for plutonium. The average of the five "dummy" injections was taken as the average dose of plutonium received by the animals in the group.

Methods of Analyses

The kidneys, liver, and spleen were prepared for analysis in essentially the same manner. The tissue was transferred to a 300-ml Kjeldahl flask and 15 ml conc HNO_3 were added. The acid was carefully boiled off until the sample partially charred. Ten ml of fuming HNO_3 were added to the contents of the flask and the sample was again boiled down until charring began. Another treatment with 10 ml of conc HNO_3 was carried out followed by 10 ml of 30 per cent H_2O_2. After the evaporation of the nitric acid and the hydrogen peroxide a white ash usually remained. If the ash was not white the entire procedure was repeated. The ash was dissolved in 4N HCl and the solution transferred to a volumetric flask and made up to volume.

The livers contained sufficient plutonium to allow a direct determination after ashing. A suitable aliquot of the solution was evaporated directly on a platinum plate. The plate was heated to a dull red to decompose traces of organic matter. The plutonium was then determined by counting the plate in a proportional alpha counter. When known amounts of plutonium were added to rat livers, the average recovery by this procedure was 90 per cent, therefore, a 10 per cent correction was applied to all liver results.

Because of the relatively small amount of plutonium in the spleen and kidneys, large aliquots of these solutions were analyzed by the cupferron extraction procedure (LA-395). When known amounts of plutonium were added to rat spleens and kidneys, the average recovery was 80 per cent. A 20 per cent correction was applied to all analyses of kidney and spleen.

The carcass, consisting of skeleton and "balance"* was placed in a glazed porcelain casserole and dried for 48 hours at 105°C. The casserole was covered with a heavy Pyrex plate and placed in a cold muffle furnace. The temperature of the furnace was then raised to 450 to 500°C over a period of 4 hours. Ashing was continued at 450 to 500°C for the next 20 hours. After cooling, the ash in the casserole was transferred to a 20-mesh sieve. The flakey white ash of the "balance" was separated from the skeleton by gently shaking the sieve.

The skeleton was transferred back to the casserole, 15 ml of conc HCl added, and the mixture allowed to stand for several hours. About 15 ml of H_2O were added and the contents of the casserole heated to boiling. The solution was transferred to a centrifuge cone and centrifuged. The supernatant liquid was transferred to a 100-ml volumetric flask. The residue was transferred back to the casserole, dried, and again ignited at 450 to 500°C until completely white. The residue was taken into solution by treating with successive portions of hot 6N HCl which were added to the volumetric flask containing the first supernatant.

The ash from the "balance" was dissolved by essentially the same method as described for the bone.

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*The "balance" represented all the carcass except skeleton, kidney, spleen, and liver.

Suitable aliquots of the bone and "balance" solutions were analyzed for plutonium by the cupferron extraction method (LA-395). When known amounts of plutonium were added to bone and "balance" ash solutions, the average recovery was 80 per cent. A 20 per cent correction was applied to all analyses of bone and "balance."

Urine samples were transferred to a 300-ml Kjeldahl flask, evaporated almost to dryness, and then digested by alternate treatment with conc HNO_3 and 30 per cent H_2O_2. The procedure was essentially that used for kidney, liver, and spleen. When a white urine ash was obtained, it was taken up in 4N HCl and transferred to a volumetric flask. Aliquots of the urine ash solution were analyzed for plutonium by the cupferron extraction procedure. Average recovery of known amounts of plutonium added to rat urine was 80 per cent. A 20 per cent correction was added to all results.

Feces samples were dried, weighed, and ground. A 5-g aliquot was transferred to a platinum crucible and carefully ignited over a low flame. The curcible was finally heated at a dull red heat until the feces ash was a greyish-white color. The ash was treated twice with 10 ml of conc HCl and evaporated to dryness each time. The residue was extracted with 10 to 15 ml of hot 4N HCl, transferred to a centrifuge cone, centrifuged, and the supernatant transferred to a 50-ml volumetric flask. The extraction was repeated. The residue was then transferred back to the platinum crucible and ignited again for one hour. The residue was fumed twice with 5 ml of conc HCl and 5 to 10 ml of conc HF. After all HF was driven off, the residue was treated with hot 4N HCl and the extract transferred to the volumetric flask. If all the residue did not dissolve after the HF treatment, it was centrifuged out and discarded after an additional treatment with 1/2 ml of aqua regia.

A suitable aliquot of the feces ash solution was analyzed for plutonium by the cupferron extraction procedure. A 20 per cent correction was applied to all feces results.

EXPERIMENTAL RESULTS

Effect of Valence State and Citrate Ion on Urinary and Fecal Excretion of Plutonium Administered Intravenously to the Rat

All laboratories concerned with the study of the physiological problems associated with plutonium have reported excretion data. Little effort has been expended, however, on establishing the effect of valence of plutonium on the excretion rate. Hamilton et al (CN-2383) did not run complete excretion curves but pooled samples for analysis at rather broad time intervals. They were primarily concerned with excretion following intramuscular administration and failed to observe any essential differences in the rate of excretion of Pu^{+3}, Pu^{+4}, and PuO_2^{+2} from the animal body.

Table 2 persents the data collected during our study of the daily urinary and fecal excretion of plutonium when administered intravenously to the rat as $PuCl_3$, $Pu(NO_3)_4$, $PuO_2(NO_3)_2$, and Pu^{+4} citrate complex. These data are presented graphically in the form of excretion curves in Figures 2 to 9. The most obvious point indicated by these results was the extremely high excretion of PuO_2^{+2} in the urine during the first day.

During the first day the urinary excretion of plutonium administered as $PuO_2(NO_3)_2$ was 7.5 per cent of the injected dose as compared to 0.33, 0.57, and 0.71 per cent for Pu^{+3}, Pu^{+4}, and Pu^{+4} citrate complex, respectively. Russell (CN-3167) observed a high urinary excretion of plutonium by the dog, during the first few hours following the intravenous administration of $PuO_2(NO_3)_2$. It seems quite possible that this high excretion rate of plutonyl plutonium during the first day is an actual physiological result of the valence state. Throughout the first 4 to 6 days the urinary excretion of plutonium injected as $PuO_2(NO_3)_2$ remained somewhat higher than the excretion when the plutonium was injected in the other forms. This time interval may be assumed to be the time required for all of the PuO_2^{+2} ions to be reduced to a lower valence. It is interesting to note that the very high urinary excretion of PuO_2^{+2}

was accompanied by a lowered fecal excretion rate. Only 5.6 per cent of the injected dose was excreted in the feces during the first four days as compared to 13.8 per cent for $PuCl_3$, 14.0 for $Pu(NO_3)_4$, and 10 per cent for Pu^{+4} citrate complex. The high fecal excretion of plutonium after injection as $PuCl_3$ or $Pu(NO_3)_4$ may be, however, a result of its higher deposition in the liver (see Table 4).

The data in Table 3 compare the urinary and fecal excretion of the various forms of plutonium at one day and at 30 days after injection. This table was prepared from the curves in Figures 2 to 9 by taking the urinary and fecal excretion values at 30 days. The ratios of fecal to urinary excretion during the first day after injection emphasizes the extent to which the excretion of plutonyl plutonium differed from the excretion of plutonium administered in other forms. In the former case the ratio was only 0.3/1 compared to 3/1 to 10/1 for the other forms. On the thirtieth day after injection there were no outstanding differences in either urinary or fecal excretion regardless of the form in which the plutonium had been administered. At this time the average daily urinary excretion was about 0.013 per cent of the injected dose. The average fecal excretion was 0.22 per cent and the average fecal to urinary excretion ratio was 16/1.

The excretion curves shown in Figures 2 to 9 emphasize the extremely high fecal excretion of plutonium by the rat. Regardless of the form in which the plutonium was injected, the fecal output 30 days after injection was 16 times as large as that in the urine. In this respect the human and the rat appear to differ widely. The ratio of fecal to urinary excretion of plutonium by the human at 30 days is at most 1/1 (unreported data obtained at this laboratory). The wide difference in the rate at which the rat and the human excrete plutonium in the feces should be considered when evaluating toxicological studies in which the rat was used as the test animal.

There were no outstanding differences in the excretion of Pu^{+4} when injected as the citrate complex and as $Pu(NO_3)_4$. In the former case, however, fecal excretion was somewhat lower during the first few day . The lower fecal excretion may be due to a lower deposition in the liver (see Table 4).

Effect of Valence State and Citrate Ion on Body Distribution of Plutonium Administered Intravenously to the Rat

The data in Table 4 show the distribution of plutonium in the rat body at 4, 16, and 32 or 48 days after intravenous injection as $PuCl_3$, $Pu(NO_3)_4$, Pu^{+4} citrate complex, and $PuO_2(NO_3)_2$. Regardless of the original form in which the plutonium was injected, the skeleton was the principal site of deposition. When the plutonium was administered as Pu^{+4} citrate and $PuO_2(NO_3)_2$, the skeletal deposition was approximately 60 per cent of the injected dose. Liver deposition was quite low when the plutonium was injected in these two forms. Four days following injection the amounts deposited in the liver were 9.1 and 9.6 per cent, respectively. When injected as $PuCl_3$ and $Pu(NO_3)_4$, the per cent of the dose deposited in the liver was much higher than when administered as Pu^{+4} citrate or $PuO_2(NO_3)_2$. Deposition in the liver 4 days after administration of $PuCl_3$ was 22.9 per cent of the injected dose. When injected as $Pu(NO_3)_4$, deposition in the liver was 39.7 per cent. Skeletal deposition of plutonium was correspondingly lower for those forms giving the higher liver content. Four days following injection of plutonium as $PuCl_3$ and $Pu(NO_3)_4$, deposition in the skeleton was 44.9 and 29.4 per cent of the dose, respectively.

No outstanding differences were observed in the deposition of plutonium in the kidney following intravenous injection of the various forms. At the end of the 4th day kidney deposition ranged from 1.36 to 2.20 per cent. At 16 days deposition in the kidney ranged from 0.67 per cent in the case of $PuCl_3$ to 1.14 per cent for $PuO_2(NO_3)_2$.

Deposition of plutonium in the spleen following intravenous injection was not greatly different for the various forms of plutonium injected. As was the case with the liver, the highest deposition in the spleen was obtained with $Pu(NO_3)_4$. When injected as $PuCl_3$, $Pu(NO_3)_4$, Pu^{+4} citrate, and $PuO_2(NO_3)_2$, the average deposition of plutonium in the spleen for the three time intervals was 1.34, 0.81, 0.61, and 0.44 per cent, respectively.

Table 2. Effect of valence state and citrate ion on urinary and fecal excretion of plutonium administered intravenously to the rat.

Sampling period days after injection	Avg.* per cent of injected dose† excreted							
	$PuCl_3$		$Pu(NO_3)_4$		Pu^{+4} citrate‡		$PuO_2(NO_3)_2$	
	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces
0–½	0.191	1.99	0.436	0.12	0.436	0.38	6.68	0.78
½–1	0.139	1.26	0.137	1.63	0.269	1.89	0.937	1.51
1–2	0.182	3.24	0.169	3.77	0.240	2.94	0.400	1.29
2–3	0.127	3.94	0.124	4.43	0.201	2.72	0.300	1.07
3–4*	0.080	3.44	0.117	4.09	0.165	2.07	0.250	0.95
4–5	0.057	3.16	0.086	2.58	0.137	1.50	0.238	1.07
5–6	0.052	2.64	0.081	2.15	0.126	1.29	0.150	0.93
6–7	0.057	2.21	0.104	1.60	0.084	1.79	0.175	1.03
7–8	0.067	2.64	0.069	1.65	0.087	1.30	0.137	0.86
8–9	0.051	1.87	0.054	1.17	0.075	1.01	0.137	0.60
9–10	0.046	1.81	0.061	1.14	0.076	0.89	0.099	0.83
10–11	0.039	1.94	0.042	0.89	0.066	0.86	0.065	0.92
11–12	0.037	1.09	0.050	0.61	0.059	0.92	0.059	0.60
12–13	0.032	1.23	0.040	0.70	0.057	0.45	0.054	0.58
13–14	0.032	1.11	---	0.58	0.054	0.54	0.054	0.58
14–15	0.029	0.82	0.042	0.63	0.047	0.56	0.051	0.37
15–16*	0.022	0.57	0.030	0.71	0.040	0.41	0.050	0.24
16–17	0.031	0.72	0.036	0.55	0.046	0.47	0.011	0.48
17–18	0.014	0.63	0.036	0.55	0.046	0.47	0.024	0.69
18–19	0.019	0.54	0.035	0.51	0.034	---	0.015	0.29
19–20	0.009	0.33	0.035	0.51	0.034	---	0.015	0.24
20–21	---	0.26	---	0.37	---	0.28	0.012	0.17
21–22	0.014	0.23	---	0.37	---	0.28	0.006	0.22
22–23	0.009	0.29	0.025	---	0.026	---	0.005	0.30
23–24	0.009	0.23	0.025	---	0.026	---	0.005	0.19
24–25	0.009	0.20	---	0.25	---	0.52	0.008	0.21
25–26	0.012	0.22	---	0.25	---	0.52	0.017	0.49
26–27	---	0.24	0.025	---	0.026	---	0.012	0.27
27–28	0.022	0.19	0.025	---	0.026	---	0.011	0.22

(Continued on next page)

Table 2. (Continued)

Sampling period days after injection	Avg.* per cent of injected dose† excreted							
	$PuCl_3$		$Pu(NO_3)_4$		Pu^{+4} citrate‡		$PuO_2(NO_3)_2$	
	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces
28–29	0.017	0.13	---	0.25	---	0.42	0.011	0.42
29–30	0.015	0.17	---	0.25	---	0.42	0.005	---
30–31	0.014	0.18	0.017	---	0.016	---	0.005	0.30
31–32	0.009	0.16	0.017	---	0.016	---	0.011	0.11
32–33			---	0.35	---	0.30		
33–34			---	0.35	---	0.30		
34–35			0.014	---	0.017	---		
35–36			0.014	---	0.017	---		
36–37			---	0.39	0.017	---		
37–38			---	0.39	---	0.32		
38–39			0.014	---	---	0.32		
39–40			0.014	---	0.014	---		
40–41			---	0.26	0.014	---		
41–42			---	0.26		0.20		
42–43			0.016	---		0.20		
43–44			0.016	---		0.36		
44–45			---	0.29		0.36		
45–46			---	0.29				
46–47			0.010	---				
47–48								

* The numbers of rats composing the average excretion values in this table were as follows:

Sampling period	$PuCl_3$	$Pu(NO_3)_4$	Pu^{+4} cit.	$PuO_2(NO_3)_2$.
Through 4th day	9	12	12	15
Through 16th day	6	8	8	9
After 16th day	3	4	4	3

† The dosages injected were $PuCl_3$ 15.2γ, $Pu(NO_3)_4$ 14.2γ, Pu^{+4} citrate 15.0γ, and $PuO_2(NO_3)_2$ 15.1γ.

‡ Pu^{+4} in a 0.3 per cent solution of $Na_3C_6H_5O_7 \cdot 5\frac{1}{2}H_2O$.

Table 3. A comparison of fecal and urinary excretion of various forms of plutonium on the first and 30th days after intravenous administration to the rat.

Cpd. and valence	% dose excreted 1st day			%dose excr. 30th day		
	Urine	Feces	F/U	Urine	Feces	F/1
$PuCl_3$	0.33	3.27	10/1	0.011	0.15	14/1
$Pu(NO_3)_4$	0.57	3.80	7/1	0.016	0.28	17/1
$PuO_2(NO_3)_2$	7.50	2.30	0.3/1	0.011	0.18	16/1
Pu^{+4} cit.	0.71	2.25	3/1	0.016	0.28	17/1

The time intervals chosen in this study (4, 16, and 32 or 48 days) were much too short to permit any significant conclusions regarding the effect of time on distribution and relocation of plutonium in the body following intravenous injection in various forms.

In general, the plutonium content of the liver, kidneys, and "balance" decreased with time. The skeleton and spleen did not show any significant changes during the short time intervals allowed for the experiment.

The data in Table 4 and the excretion curves shown in Figures 2 to 9 suggest that fecal excretion of plutonium was higher for those forms giving higher deposition in the liver, namely $PuCl_3$ and $Pu(NO_3)_4$.

The results reported in Table 4 on body distribution of plutonium injected intravenously into the rat agree qualitatively with those reported by Hamilton (CN-2383). Quantitatively the agreement is poorest with regard to the deposition of plutonium in the liver and skeleton. Four days following intravenous injection of $PuCl_3$, $Pu(NO_3)_4$, and $PuO_2(NO_3)_2$, he reported liver deposition of 38, 73, and 29 per cent, respectively, compared to 22.9, 39.7, and 9.1 per cent found in this study. Four days following injection he found deposition in the skeleton was 33.5, 14.4 and 46.0 per cent, respectively, for plutonium injected in the three valence states. In this study the respective values for skeletal deposition were 44.9, 29.4, and 56.5 per cent. The body distribution of plutonium 16 days following intravenous injection as $PuO_2(NO_3)_2$ agrees quite well with that reported by Hamilton's group (CN-2740).

The data presented in Table 4 indicate that complexing Pu^{+4} with citrate ion drastically lowered the amount of plutonium deposited in the liver following intravenous injection. This result supports the postulate that high deposition in the liver following intravenous injection may result from hydrolysis of the plutonium at the pH of the blood. The colloidal particles formed by hydrolysis may then be filtered out by the reticulo-endothelium of the liver (CN-2383). The Chicago laboratories do not concur in this belief. Their results have not shown a pronounced affect of citrate on liver deposition of intravenously injected plutonium (CN-2653).

Effect of Size of Dose on Metabolism of Plutonium in the Rat

If urinary excretion is to provide a basis for determining the degree of exposure of workers to plutonium, the metabolism of this material in the body must be independent of the size of dose. The metabolism must be essentially the same whether the body content should result from a single large dose or from several small ones. The following experiment was devised to study the effect of size of dose on the excretion and body distribution of plutonium when administered intravenously to the rat.

Table 4. Effect of valence state, citrate ion and time after injection on metabolism of plutonium administered intravenously to the rat.

Days after injection	Tissue or excretion	Avg.* per cent of injected dose.† $PuCl_3$	$Pu(NO_3)_4$	Pu^{+4} cit‡	$PuO_2(NO_3)_2$
	Liver	22.92	39.69	9.56	9.11
	Spleen	0.73	1.19	0.67	0.51
	Kidneys	2.20	1.36	1.64	1.91
4	Skeleton	44.91	29.43	56.93	56.54
	Balance	13.54	11.88	13.70	11.21
	Urine	0.90	0.93	1.34	7.89
	Feces	17.15	15.11	10.89	5.71
	Total	102.3	99.6	94.7	92.9
	Liver	7.04	26.14	4.17	3.37
	Spleen	0.96	1.40	0.60	0.48
	Kidneys	0.67	0.74	0.79	1.14
16	Skeleton	42.28	30.88	60.30	58.50
	Balance	11.95	7.28	10.15	7.58
	Urine	1.35	1.69	2.15	9.18
	Feces	37.61	26.03	20.83	12.91
	Total	101.8	94.2	99.0	93.2
	Liver	5.38			2.78
	Spleen	0.73			0.33
	Kidneys	0.50			0.73
32	Skeleton	51.81			56.84
	Balance	7.86			9.46
	Urine	1.20			9.30
	Feces	33.86			20.54
	Total	101.3			99.9
	Liver		21.84	2.70	
	Spleen		1.43	0.57	
	Kidneys		0.43	0.36	
48	Skeleton		31.88	60.45	
	Balance		5.21	7.85	
	Urine		2.15	2.85	
	Feces		39.65	29.68	
	Total		102.6	104.4	

* Each value is an average of results obtained for the tissues or excrement of 3 rats in case of $PuCl_3$, 4 rats in case of $Pu(NO_3)_4$ and Pu^{+4} citrate and 6 rats in case of $PuO_2(NO_3)_2$.

† The dosages injected were $PuCl_3$ 15.2γ, $Pu(NO_3)_4$ 14.2γ, Pu^{+4} citrate 15.0γ, and $PuO_2(NO_3)_2$ 15.1γ.

‡ Pu^{+4} in a 0.3 per cent solution of $Na_3C_6H_5O_7 \cdot 5\frac{1}{2}H_2O$.

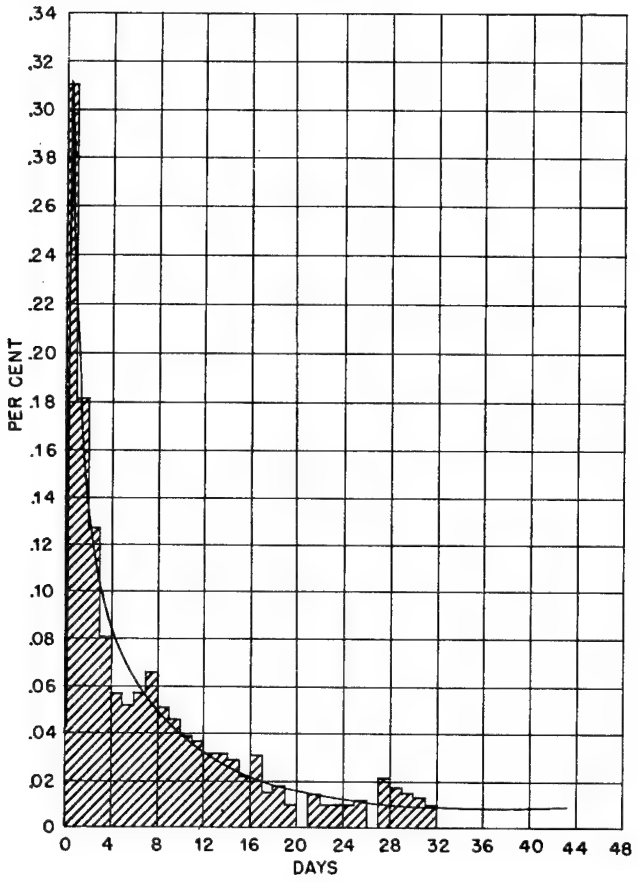

Figure 2. Urinary excretion of Pu by the rat, 15.2 r Pu administered intravenously as $PuCl_3$— per cent of dose excreted per day.

Five groups of mature male rats were injected with 0.032γ (2250 c/m), 1.1γ, 5.3γ, 15γ, and 52γ of plutonium, respectively. The plutonium was administered as Pu^{+4} citrate complex in a solution 0.5 per cent with sodium citrate. The pH of the solution was approximately 6 and the volume injected was kept constant—0.35 ml. Urine and feces samples were collected daily for six days and analyzed for plutonium. At the close of the sixth day following injection the animals were sacrificed and their tissues analyzed.

The data in Table 5 show the relationship between size of dose and the daily urinary and fecal excretion of plutonium injected intravenously into the rat. The per cent of the injected dose excreted per day in both feces and urine was not significantly affected by the size of the dose. In fact, the agreement in per cent excreted for the various sized doses was remarkably close. At the end of the sixth day after injection the spread in total fecal excretion was from 8.4 to 12.8 per cent of the dose. The spread in total urinary excretion was from 1.6 to 2.1 per cent.

The distribution of plutonium in the animal body was likewise unaffected by the size of the injected dose (Table 6). The skeleton was the principal site of deposition. Approximately 60 per cent of the injected plutonium was found in the skeleton regardless of the size of the dose. The results for skeletal deposition ranged from 56.1 to 60.5 per cent for all doses.

The per cent of the dose present in the total blood volume was also quite constant. The range in blood content for the various dosages six days following injection was 1.0 to 1.5 per cent. The amount of plutonium deposited in the liver ranged from 7.8 to 10.4 per cent.

Metabolism of Plutonium Administered Orally to the Rat

Absorption of orally administered plutonium is quite low (CN-2383), MUC RRS-553, MUC KSC-520) — only about 0.01 per cent of the total administered dose. Because of the low absorption from the gastro-intestinal tract, little information is available regarding distribution in the body of plutonium absorbed via this route. Absorption of plutonium from the lung was enhanced by the presence of a complexing ion, i.e., citrate (AM-1653). The following experiment was set up to determine the

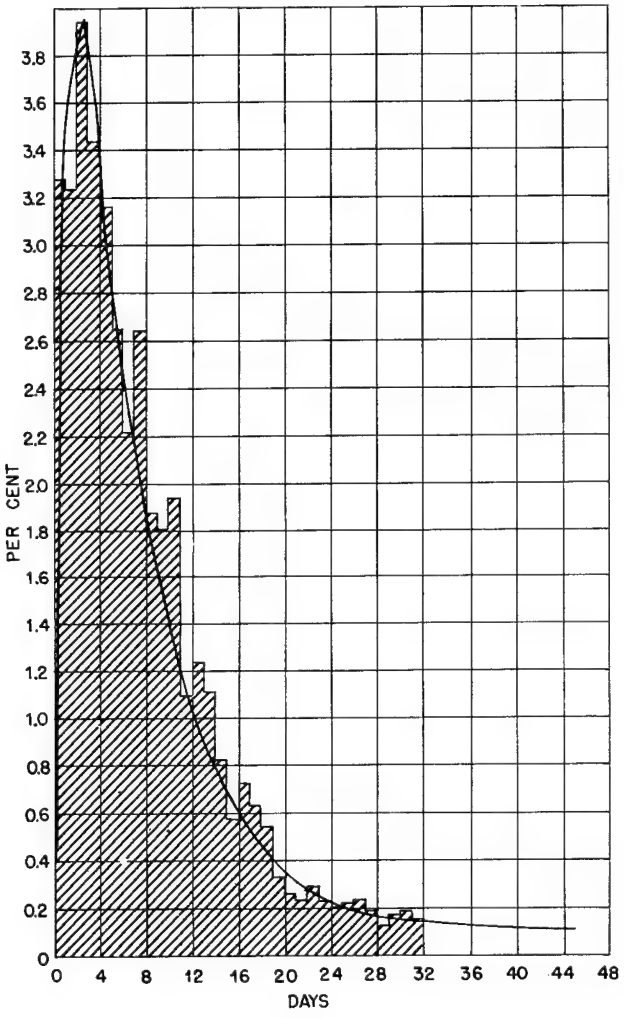

Figure 3. Fecal excretion of Pu by the rat, 15.2 r Pu administered intravenously as $PuCl_3$ — per cent of dose excreted per day.

Table 5. Daily excretion of intravenously administered plutonium* in relation to the size of dose (rat).

Period after inj.(days)	Size of dose and avg.† per cent of dose excreted in urine and feces									
	0.032γ		1.1γ		5.3γ		15.0γ		52.0γ	
	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces
0.0–0.5	0.609		0.665	1.74	0.467	0.85	0.436	0.38	0.733	2.06
0.5–1	0.312		0.218	0.88	0.476	1.57	0.269	1.89	0.189	1.11
1–2	0.338		0.265	2.04	0.419	2.51	0.240	2.94	0.321	2.13
2–3	0.276		0.133	1.37	0.220	1.71	0.201	2.72	0.244	1.89
3–4	0.181		0.151	0.81	0.165	1.13	0.165	2.07	0.208	1.42
4–5	0.176		0.139	0.80	0.104	1.30	0.137	1.50	0.199	1.40
5–6	0.176		0.139	0.80	0.104	1.30	0.126	1.29	0.199	1.40
Total	2.07		1.71	8.44	1.95	10.37	1.58	12.79	2.09	11.41

* Plutonium was injected as the +4 citrate complex prepared from $Pu(NO_3)_4$ by making the solution 0.5 per cent with $Na_3C_6H_5O_7 \cdot 5\frac{1}{2}H_2O$. The pH of each solution was approximately 6.

† Each value is the average of results of 3 rats except for the 15γ dose in which case each value is an average of results of 12 rats.

Table 6. Effect of size of dose on metabolism of plutonium* administered intravenously to the rat.†

Organ or excretion	Size of inj. dose and avg.‡ per cent of dose per organ				
	0.032γ	1.1γ	5.3γ	15γ	52γ
Liver	7.79	9.32	10.43	9.56	9.39
Spleen	0.66	0.44	0.45	0.67	0.49
Kidneys	1.33	1.22	1.22	1.64	1.46
Skeleton	56.12	57.30	60.46	56.93	60.38
Balance	18.34	14.30	14.39	13.70	14.81
Blood	1.50	1.25	1.00	--	1.10
Urine	2.07	1.71	1.95	1.58	2.09
Feces	9.18	8.45	10.37	12.79	11.41
Total	97.0	94.0	100.3	96.9	101.1

* Plutonium was injected as the +4 citrate complex prepared from $Pu(NO_3)_4$ by making the solution 0.5 per cent with $Na_3C_6H_5O_7 \cdot 5\frac{1}{2}H_2O$. The pH of each solution was approximately 6.

† All animals were sacrificed at the end of the 6th day.

‡ Each value is the average of results from 3 rats. Values for the 15 γ dose are an average of results from 12 rats.

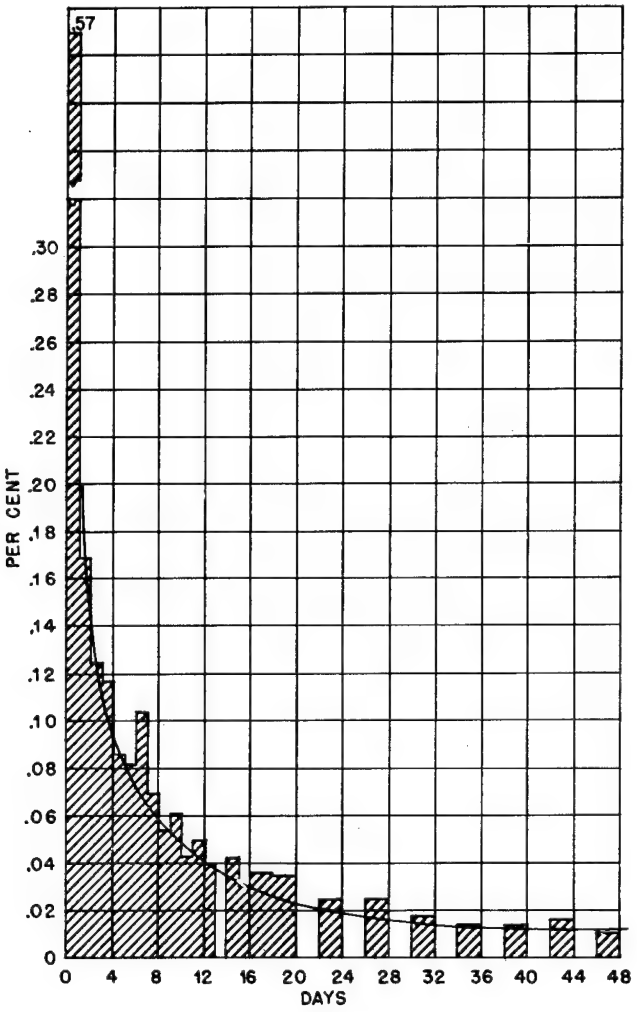

Figure 4. Urinary excretion of Pu by the rat, 14.2 r Pu administered intravenously as $Pu(NO_3)_4$ — per cent of dose excreted per day.

absorption and body distribution of plutonium when administered orally to rats simultaneously with a large excess of sodium citrate.

Three young male rats weighing 200 g each were selected for each group. Group 1 was given 46γ of Pu^{+4}, group 2 was given 198γ Pu, group 3 was given 488γ Pu, and group 4 was given 2000γ. The first three dosages were administered in a 5 per cent solution of $Na_3C_6H_7 \cdot 5\frac{1}{2}H_2O$. Through mistake, the 2000γ dose was given in a 0.5 per cent solution of sodium citrate. All dosages were administered by giving one fifth of the total dose per day for five consecutive days. The volume of solution given per was 2.5 ml.

Four days after the last administration of plutonium the animals were sacrificed and the livers, skeleton, and "balance" analyzed.

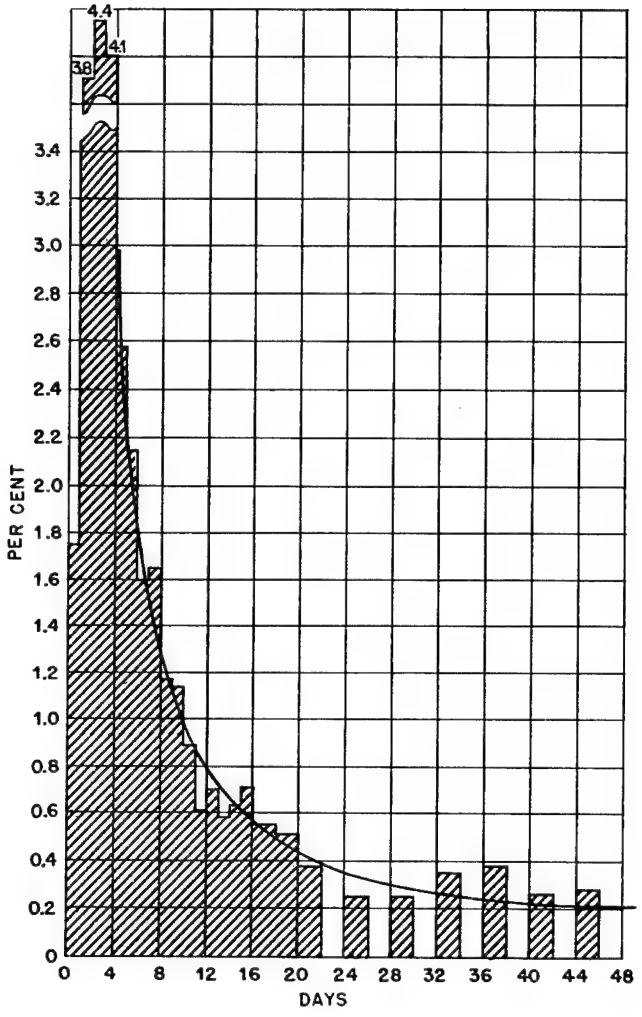

Figure 5. Fecal excretion of Pu by the rat, 14.2 r Pu administered intravenously as $Pu(NO_3)_4$ — per cent of dose excreted per day.

The results are given in Table 7. These results indicate that absorption of plutonium from the gastro-intestinal tract was increased by the simultaneous administration of a large excess of sodium citrate. The increase in absorption, however, was not great. Only about 0.3 per cent of the plutonium was absorbed when given in a 5 per cent solution of sodium citrate.

The amount of plutonium administered, and the strength of the sodium citrate solution used did not significantly affect the per cent of the absorbed dose deposited in the liver and skeleton. If the amount of absorbed plutonium that was excreted in urine and feces during the time of the experiment is disregarded, the average liver deposition for all dosages was 7.3 per cent of the amount absorbed. The average skeletal deposition under these conditions was 78.7 per cent of the absorbed material. These results indicate that the liver and skeletal deposition of plutonium absorbed from the gastro-intestinal tract was essentially the same as the deposition when the plutonium was injected intravenously

as Pu^{+4} citrate complex or as $PuO_2(NO_3)_2$. A similar comparison was made between the deposition of plutonium absorbed from an intramuscular injection and plutonium injected intravenously as $PuO_2(NO_3)_2$ (CN-2740).

General consideration of all results reported to date seems to indicate that regardless of valence state, the deposition of plutonium absorbed slowly into the body (as is the case after oral, intramuscular, or intrapulmonary administration, etc.) is similar to the deposition following intravenous injection of $PuO_2(NO_3)_2$ or Pu^{+4} citrate complex.

Table 7. Absorption and body distribution of plutonium administered orally* to the rat.

Total dose (γ)	% dose absorbed	% of abs. Pu† per organ		
		Liver	Skeleton	Balance
46	0.34	7.8	70.8	21.4
198	0.32	8.2	81.7	10.1
488	0.25	3.7	88.1	8.2
2000	0.06*	9.4	74.2	16.3
Avg.	--	7.3	78.7	14.0

* The first three doses were administered in 5 per cent solution of $Na_3C_6H_5O_7 \cdot 5\frac{1}{2}H_2O$. The 2000$\gamma$ dose through mistake was administered in 0.5 per cent citrate solution. All doses were administered by giving one-fifth of the total amount of plutonium per day for five days.

† The amount of the absorbed plutonium excreted in feces and urine during the experimental period was neglected. The animals were sacrificed ten days after the begining of the experiment.

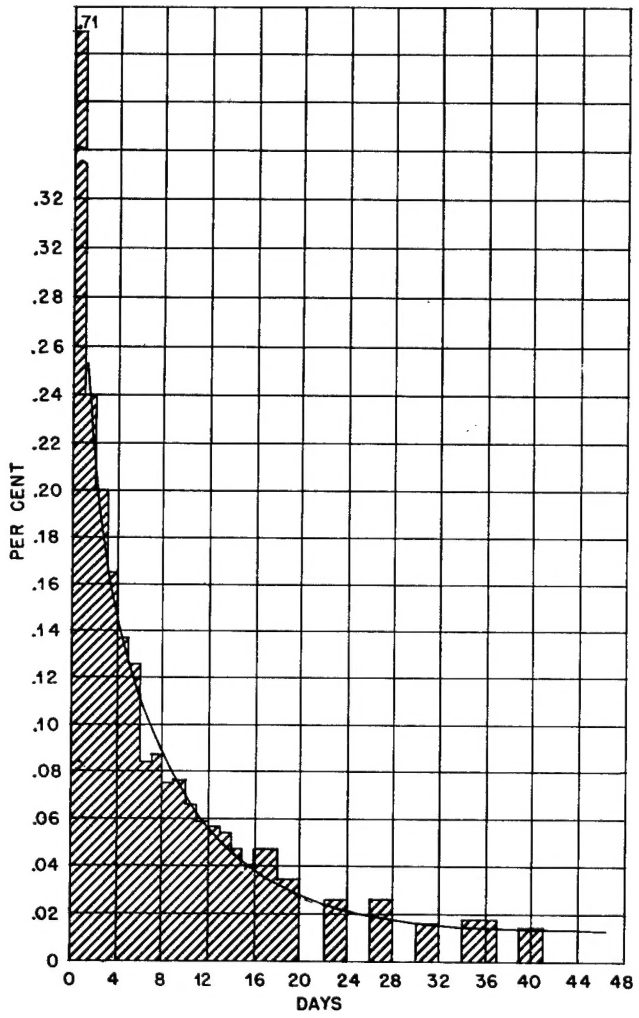

Figure 6. Urinary excretion of Pu by the rat, 15.0 r Pu administered intravenously as Pu^{++} citrate complex — per cent of dose excreted per day.

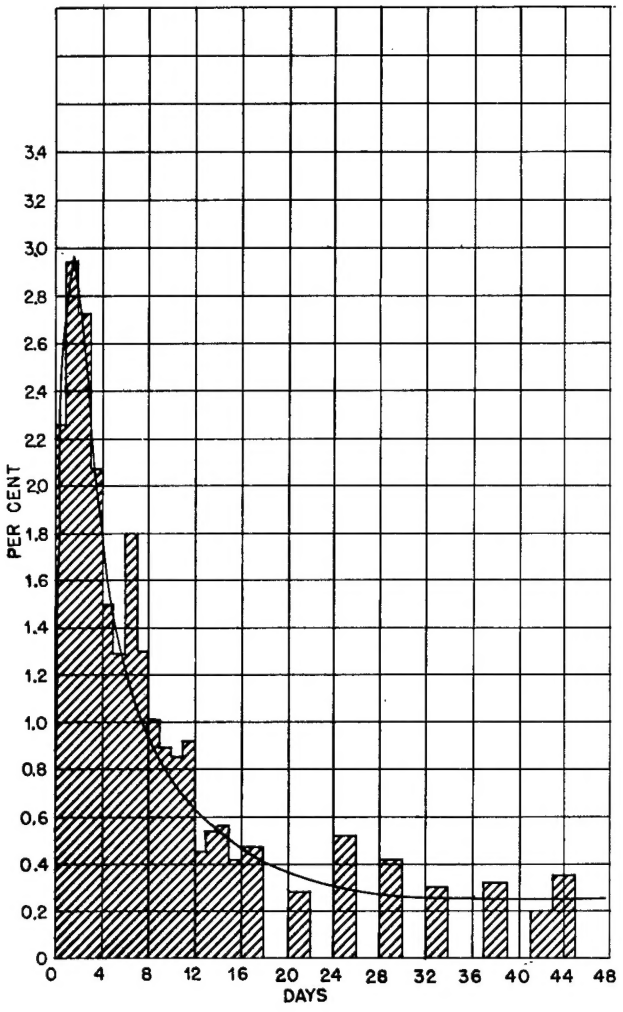

Figure 7. Fecal excretion of Pu by the rat, 15.0 r Pu administered intravenously as Pu^{++} citrate complex—per cent of dose excreted per day.

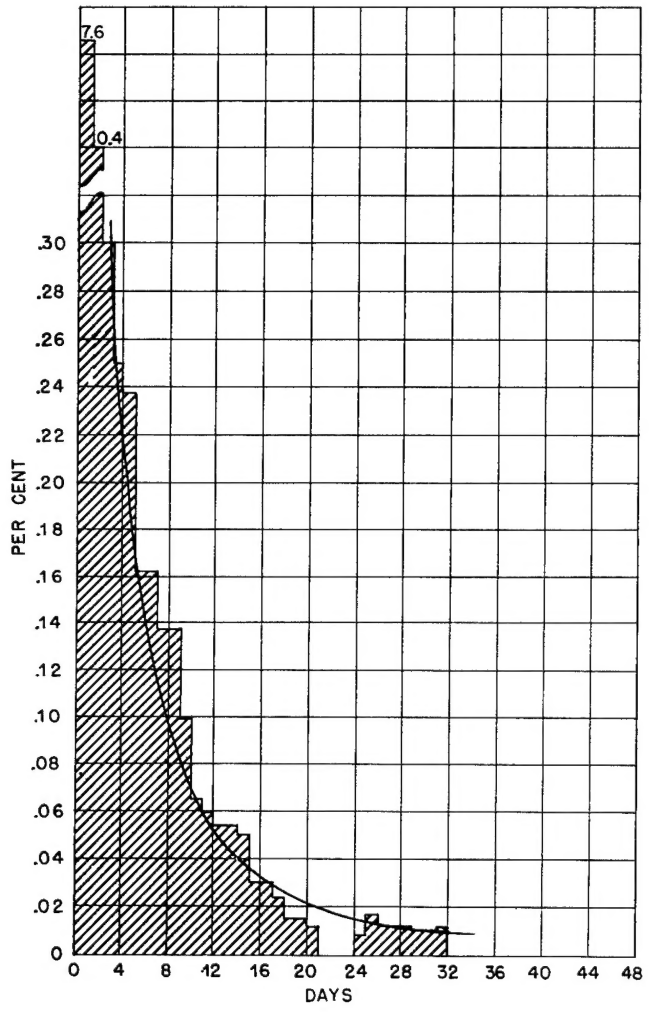

Figure 8. Urinary excretion of Pu by the rat, 15.1 r Pu administered intravenously as $PuO_2(NO_3)_2$ — per cent of dose excreted per day.

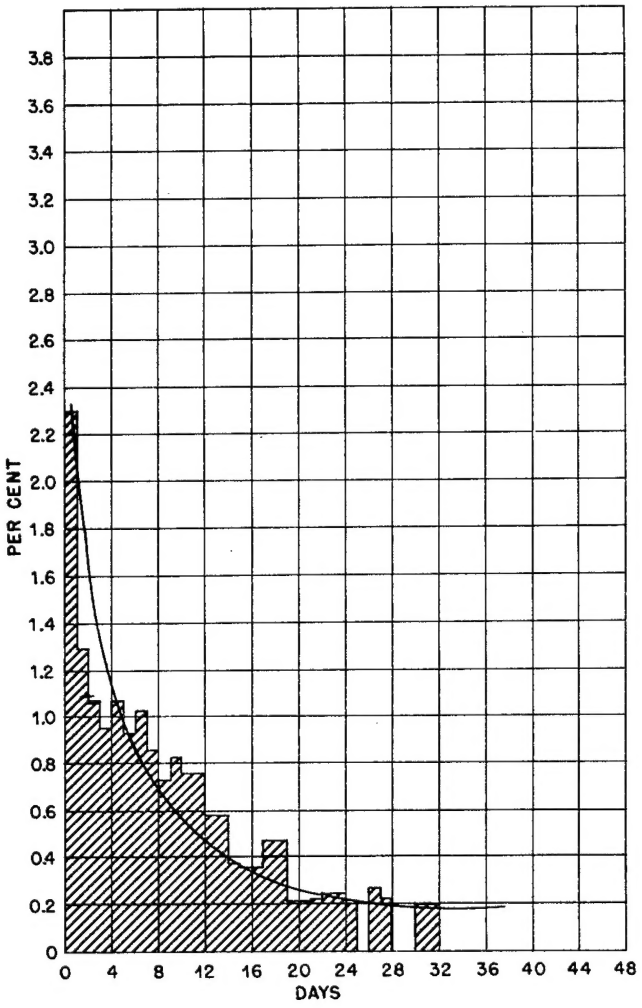

Figure 9. Fecal excretion of Pu by the rat, 15.1γ administered intravenously as $PuO_2(NO_3)_2$ — per cent of dose excreted per day.